Comparative analysis of cervical disease progression in HIV positive women receiving ART and those not receiving ART in Cape Town, South Africa, from 2002 to 2011

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DECLARATION

I, Janis Paulsen, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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For my parents who have given me everything.

Abstract

Human papilloma virus (HPV) has been shown to be a necessary cause for the development of cervical cancer. Cervical cancer is a progressive disease, going through pathologically distinct stages ranging from normal cytology to malignancy. Precancerous lesions (low and high grade squamous epithelial lesions (SIL)) are detectable and treatable. In addition, women who are infected with human immunodeficiency virus (HIV) have been shown to be more likely to be infected with HPV than their HIV negative counterparts. Cervical cancer precancerous lesions have also been shown to be more commonly detected in HIV positive than in HIV negative women. Cervical cancer is classified as an acquired immunodeficiency syndrome (AIDS)-defining illness.

The advent of antiretroviral therapy (ART) has seen an increase in the life span of HIV positive women. The use of ART has also seen a decline in the incidence of other AIDS-defining illnesses, such as Kaposi sarcoma and non-Hodgkin's lymphoma. Thus, a reduction in the incidence of cervical cancer among HIV positive women using ART is expected. However, the research thus far has been controversial, with some studies showing an increase in the incidence of cervical cancer in HIV positive women, others showing a decrease and still others showing no difference in the incidence. The aim of this project was to determine the incidence of precancerous lesions in HIV positive women receiving ART and to compare this to HIV positive women who are not receiving ART.

Four hundred HIV positive women were enrolled into the MACH (Management of abnormal cytology in HIV-positive women) study from two primary health care clinics and one colposcopy clinic, in Cape Town, South Africa. Enrolment into the study was dependent on cervical screening naivety. At the time of enrolment, ART was not available to these women. However, as ART was rolled out in South Africa, women meeting ART eligibility criteria (at the time, CD4 count ≤ 200 cells/ml) were started on ART. Women were followed up at 6 monthly intervals, at which point cervical smears were taken and appropriate referral for colposcopy was done. Composite diagnoses were generated using both the visual colposcopy result and the histology result, where available. Women were dichotomized into ART and non-ART groups. Survival analysis was used to determine the time taken from normal cytology to incident SIL. Entrance into the survival analysis was dependent on a baseline cytological/histological diagnosis of normal. Thus 177 out of the 400 women were included. Follow up was censored at the first of SIL detection and treatment for SIL. A Cox model was built to determine the hazard for SIL development. This hazard was adjusted for ART status, CD4 count and age. CD4 count was treated as a time varying covariate.

Women older than 40 years had a showed a 58% reduction in the progression of cervical disease from normal to SIL compared to women younger than 40 years. Women with baseline CD4 counts > 500 cells/ml had a 67% decrease in SIL development compared to those with a baseline CD4 count ≤ 200 cells/ml. Similarly, those with CD4 counts between 201 and 500 cells/ml showed a 35% reduction in cervical disease progression.

The unadjusted incidence rate of SIL in women not receiving ART was 28.5 per 100 person years and in women who had initiated ART it was 30.82 per 100 person years. There was therefore no difference in the incidence rate of SIL between the 2 groups (RR=1.08; 95% CI 0.64-1.75).

The unadjusted hazard ratio for progression to SIL was 1.3 (0.5-3.2) for those receiving ART compared to those not receiving ART. Adjusting for CD4 count and age, the hazard ratio was 0.9 (0.4-2.5). The adjusted hazard ratio for SIL development with a CD4 count > 200 cells/ml was 0.5 (0.2-0.9) compared to a CD4 count ≤ 200 cells/ml. The adjusted hazard ratios for age 30-39 years and ≥ 40 years were 0.9 (0.6-1.5) and 0.4 (0.2-0.9) respectively, compared to age < 30 years.

ART has been scaled up and since the start of its roll out, the eligibility criteria has changed from CD4 count ≤ 200 cells/ml to CD4 count ≤ 350 cells/ml. The longevity of HIV positive women can approach that of HIV negative women if ART initiation is started early enough. In light of this and of the fact that cervical lesions are more frequently detected in HIV positive women, cervical screening and referral services should be strengthened.

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PART A: Protocol

Protocol for proposed project: Comparative analysis of cervical disease progression in HIV positive women receiving ART and those not receiving ART in Cape Town, South Africa, from 2002-2011.

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Protocol Summary

Invasive cervical cancer is considered an acquired immune deficiency syndrome (AIDS)-defining illness.[1] The odds of invasive cervical cancer (ICC) in human immunodeficiency virus (HIV)-positive women has been shown to be 6.5 times that in HIV negative women, while the odds of cervical intraepithelial neoplasia (CIN) 2 and 3 in HIV positive women is 10.4 times that seen in HIV negative women.[2] Of women aged 15-49 years old, 18.5 % are HIV positive.[3] This represents a significant number of women at increased risk for development of cervical lesions and ICC. The advent of antiretroviral therapy (ART) has resulted in a decrease in the incidence of other AIDS-defining illnesses; however the impact of ART on the incidence of ICC in HIV positive women remains controversial. In this study 400 HIV positive women have been enrolled and cervical cytology and/or histology diagnoses were obtained at 6 monthly intervals. The rate of cervical disease progression will be determined using survival analysis and the rates between those who initiate ART use and those who do not will be compared and a rate ratio will be calculated. Other explanatory variables to be analysed include age, baseline CD4 count, baseline viral load and baseline human papilloma virus (HPV) status. Women were deemed HPV positive if they tested positive for high risk HPV strains and HPV negative if they tested negative for high risk HPV strains. In addition, Cox proportional hazards ratios will be determined for the risk of SIL. Crude and adjusted hazard ratios will be generated for the effect of ART, CD4 count and age on SIL incidence.

Introduction

Background

South Africa has an estimated human immunodeficiency virus (HIV) prevalence of 10.2%, one of the highest in the world and has the largest HIV epidemic in the world, with an estimated 5.5 million people infected.[3] The prevalence of (HPV) among South African women is high, with an estimated 15.5% of women with normal cervical cytology being positive for HPV.[4] This is higher than the world prevalence of 10%.

Furthermore, it has also been found that women who are HIV positive are more likely to be infected with HPV. A study comparing the prevalence of HPV between HIV positive and HIV negative women found that HIV positive women were more likely to have high, low and intermediate risk HPV infections (1.8, 2.1 and 2.7 times, respectively) than their HIV negative counterparts.[5] Similarly, Ng'andwe et al[7] reported a 1.8 fold increase in high risk HPV infections in HIV positive women compared to their HIV negative counterparts, in Zambia. There was also a 2.1 and 2.7 fold increase in the likelihood of intermediate and low risk HPV infections in HIV positive women. In a Cameroonian cohort, it was found that HIV positive women were more likely to have multiple and high risk HPV subtypes compared to their HIV negative peers.[7]

In developed countries the incidence of cervical cancer has been reduced due to the establishment of good screening systems. However in the developing world setting up adequate screening and diagnostic systems has proved challenging in the face of weak infrastructure, resulting in an unnecessarily high incidence of cervical cancer. This poor screening and diagnostic system further compounds the phenomenon of a strong association found between HIV positivity and incidence and persistence of HPV infection, as well as the higher incidence and prevalence of squamous intraepithelial lesions (SIL).[8] Furthermore, the increase in access to antiretroviral therapy heralds an age in which there is an increase in the lifespan of these HIV positive women, further emphasising the importance of the need for effective cervical screening and diagnostic systems.

Six et al[9] found that the one-year prevalence in a cohort of HIV negative and positive women was over 4 times higher in the HIV positive women than in the HIV negative women. The incidence of SIL was 5.5 times higher in the HIV positive women than in their HIV negative counterparts and progression from low grade SIL (LSIL) to high grade SIL (HSIL) occurred in 38% of HIV positive women, but not in the HIV negative controls at all over the one year follow-up period. Massad, et al[10] found that over a 5 year follow up period 73% of all HIV positive study participants presented with at least 1 cytological abnormality of the cervix compared to 42.3% of all HIV negative participants. HIV status was found to be a predictor of cytological abnormalities and the risk ratio of LSIL between HIV positive women and HIV negative women was 4.

The advent of the ART era saw a drop in incidence and mortality due to AIDS-defining illnesses, such as Kaposi Sarcoma[11] and non-Hodgkin's Lymphoma[12] in Europe. However, the same cannot be said for cervical cancer. Various studies have yielded conflicting results regarding the association between ART and cervical disease progression, and as a result it is still unclear whether the use of ART results in a decrease in cervical neoplasias. Some of the evidence points to a decrease in the occurrence of cervical dysplasia, however other evidence indicates that there is no change in prevalence of SIL among HIV positive women on ART.

Heard et al[13] found that the prevalence of SIL among HIV positive women decreased significantly after a median period of 5 months after starting ART in France. A cohort study by Minkoff et al[14] in USA found that women on ART were 32% less likely to have progression of cervical disease over 6 months. In Italy, Soncini et al[15] found that over a 37 month follow up period, women on highly active ART (ART) had a 70% reduction in the risk for developing CIN compared to HIV positive women not on ART, over the same time period.

On the other hand, monitoring of the rate of progression of cervical disease in 163 HIV positive women in Italy over a mean period of 15.4 months[16] showed that there was no difference between women on ART and women not on ART. In addition, Schuman et al[17] reported that ART-use did not result in a decreased risk of incident SIL in US women.

Aim

The aim of this study is to compare the rate of cervical disease progression in HIV positive women who are have initiated ART use to that in HIV positive women who have not initiated ART use.

Objectives

The specific objective is to compare the rate of cervical disease progression between women receiving ART and women not receiving ART. The secondary objectives are to stratify the rates of disease progression by baseline CD4 count, baseline viral load, baseline HPV status and age; and also to determine the hazard for SIL development using more than one of the explanatory variables simultaneously.

Methods

Study design

This is a secondary analysis of data generated in the Management of Abnormal Cytology in HIV-infected women (MACH) cohort study.[18] Women were enrolled from primary health care clinics or from a colposcopy clinic in Cape Town, South Africa. Women were enrolled within a 10 month period between March 2002 and January 2003. Women were followed up for a median of 2.5 years, although 39 of the 400 (just under 10%) were followed up for 9 years. Those women who were not followed up to the final visit date (the 18th biannual visit) were either classified as lost to follow up or confirmed as dead.

Study population

Four hundred women were enrolled into the study from two primary health care clinics and one colposcopy clinic, to which they were referred if they had an abnormal Pap smear. Women met the inclusion criteria if they were HIV positive and previously unscreened for cervical disease. This included the women who were recruited from the colposcopy clinic, who also had to be previously unscreened at the clinic that referred them for colposcopy.

Measurement

The outcome of interest in this study is SIL. SIL will include both high grade and low grade SIL. Screening of the cervix uteri was undertaken every 6 months and a cervical cytological diagnosis was obtained. Women were referred for colposcopy if the cytological diagnosis indicated this. If an acetowhite lesion was seen under the colposcope, a punch biopsy of this area was obtained. This sample was used to make a histologic diagnosis.

If both a visual colposcopy and a histological diagnosis are available, a composite diagnosis will be constructed and used in analysis. CIN2 and CIN3 will be classified as HSIL regardless of the visual colposcopy result. CIN1 will be classified as HSIL if

the visual colposcopy is HSIL and LSIL if the visual colposcopy is LSIL or normal. If only a cytological diagnosis is available, this will be used in analysis.

Other variables to be used in the analysis are:

- CD4 count: this will be used as a categorical variable.
- HPV status: this will be used as a categorical variable.
- Age: this will be used as a categorical variable.
- Viral load: the log of viral load will be used as a categorical variable.
- ART status: this will be used a categorical variable.

Data management and analysis

The database is maintained by staff in the colposcopy clinic where the participants receive their consultations. The data is stored using Microsoft Access. The data will be transferred from Access to Stata, which will be used for all data management including data cleaning, manipulation and analysis.

The data will be analysed using survival analysis. The event of interest will be squamous cell intraepithelial lesions and thus I will be evaluating the time from entry into the study until the first time this event is experienced. Follow up will be censored at the first of SIL diagnosis and receiving treatment for cervical disease.

Kaplan Meier survival plots will be generated, and the principle outcome to be evaluated is the effect of ART on the time to SIL. Therefore, survival will be stratified by ART status. The analysis will also be stratified by baseline CD4 count, baseline viral load, baseline HPV status and age.

A Cox proportional hazards model will be built in order to determine the hazard ratios for SIL for each covariate. This type of model is appropriate for dealing with loss to follow up, a phenomenon to which longitudinal cohort studies are more often than not subject to. In addition it also does not make any underlying assumption about the baseline hazard of individual participants and assumes that the hazards remain proportional and constant over time.

Ethics

Human ethics approval for this study was obtained from the University of Cape Town Health Science Human Research Ethics Committee. The data was routinely collected each time a participant visited the clinic. Therefore analysis of this data will not result in any foreseeable harm to the participants. Personal identifiers such as names and South African identity numbers have been removed from the dataset, so therefore confidentiality of participants is ensured.

The results of this study will be disseminated by submission of abstracts to relevant conferences for presentation, as well as submission of abstracts to peer reviewed journals for publication. This should ensure adequate dissemination of the study results at both the national and international level.

Study limitations

The validity of the study is dependent on the accurate entry of data. As far as possible, data entries will be scrutinized and the plausibility considered. For example, dates will be checked for errors such as dates occurring in the future or occurring in the past (in the case of participant consultation dates).

Self-report biases could affect the baseline demographic comparisons between different groups. For example, questions requiring recall on the part of the participant could be incorrectly answered, resulting in inflated or deflated differences between comparison groups.

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Part B: Literature Review

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Introduction

Cervical cancer is the second commonest cancer among South African women.[1] Cervical disease is the progressive alteration of the cytology of the cervix from that of normal cytology to cervical cancer. There are many risk factors for cervical cancer, including smoking,[2] *Clamydia trachomatis* infection,[3] long term use of oral contraceptives,[4] and early age at sexual debut, increased number of sexual partners over a lifetime and sexual partners with high risk contacts.[5] However, a necessary cause for cervical disease is infection with human papilloma virus (HPV).[6] The disease takes many years to progress and goes through multiple intermediate stages. Following infection, the cervical cytology will change from normal to low grade squamous intraepithelial lesions (LSIL). It is notable that at this stage the cytology may spontaneously regress to a normal cytological state.[7] However, further progression can occur in which the cervical lesions develop into high grade squamous intraepithelial lesions (HSIL). Further progression from HSIL will result in a malignant cervix, or cervical cancer.

Multiple HPV types exist. Different subtypes are responsible for different types of pathological manifestations in different tissue types. HPV-16 and HPV-18 are most commonly associated with a high risk for cervical cancer.[8] In addition, HPV 16 is also associated with anal intraepithelial neoplasia and anal cancer as well as cancers of the head and neck.[9, 10] Others, such as HPV-6 and HPV-11 are more closely associated with genital warts. [11, 12] Thus it is only infection in the cervix with high risk types for cervical lesions which is of concern for cervical cancer.

Two different vaccines exist which elicit immune responses to these high-risk HPV types. These are Gardasil[13] and Cervarix[14]. Gardasil is quadrivalent and targets HPV 16 and 18 as well as the strains associated with genital warts, while Cervarix is a bivalent vaccine and only targets HPV 16 and 18. Administration of these vaccines is aimed at females who are not yet sexually active or who have only recently become sexually active[15]. It is a form of primary prevention. However, these vaccines are expensive and usually not available through a public health care system. In South Africa, the vaccine is currently being administered to girl learners aged 9 and in grade 4. This is being implemented in the form of a mass vaccination campaign and will take place every year from 2014 to 2016. Thereafter the process will be reviewed and evaluated, in order for consideration for inclusion of the vaccine into the Expanded Programme on Immunisation.[16]

In the absence of primary prevention, secondary prevention in the form of a screening system is the principle way in which precancerous lesions are identified. During screening, a cervical smear, also known as a Papanicoloau smear, is collected. The smear is stained and examined under a microscope for identification of cervical lesions. High grade lesions or worse are eligible for a diagnosis by colposcopy, in which the cervix is examined using a colposcope. If required, a biopsy may be taken and sent for histological analysis, or the diagnosis may be made visually. For HSIL, procedures such as long loop excision of the transformation zone (LLETZ) or cryotherapy will be performed to remove the pathological tissue and thus preventing progression to cervical cancer.[17]

The South African guidelines on cervical smears and colposcopy[18] state that women aged 30 years and older should have a cervical smear done once every 10 years (i.e. 3 per lifetime). The guidelines for follow up of different smear outcomes are as follows:

- Normal: return for a cervical smear in 10 years.
- LSIL: return for a cervical smear after 1 year. If after a year the result is normal, follow up is per protocol. If the result is another LSIL, the woman is to be referred for colposcopy.
- HSIL or worse: the woman is to be referred for colposcopy.

In South Africa, the processes of screening and diagnosis occur at two different levels of health care delivery, namely primary and tertiary levels respectively. Women attend the primary health care facilities for a cervical smear to be taken. The smear is sent to a laboratory for analysis and based on the outcome, the woman may or may not be referred to a colposcopy clinic. Often times, the poor follow up of patients at the primary level results in many women who require colposcopy failing to attend the colposcopy appointment at tertiary level. Regarding cervical and colposcopy services, poor follow up refers to poor recording of patient information such as address and telephone numbers, failure to action the recall of patients who require further treatment, etc. Figure 1 shows the link between primary and tertiary cervical services and cervical smear outcomes and tertiary treatment.

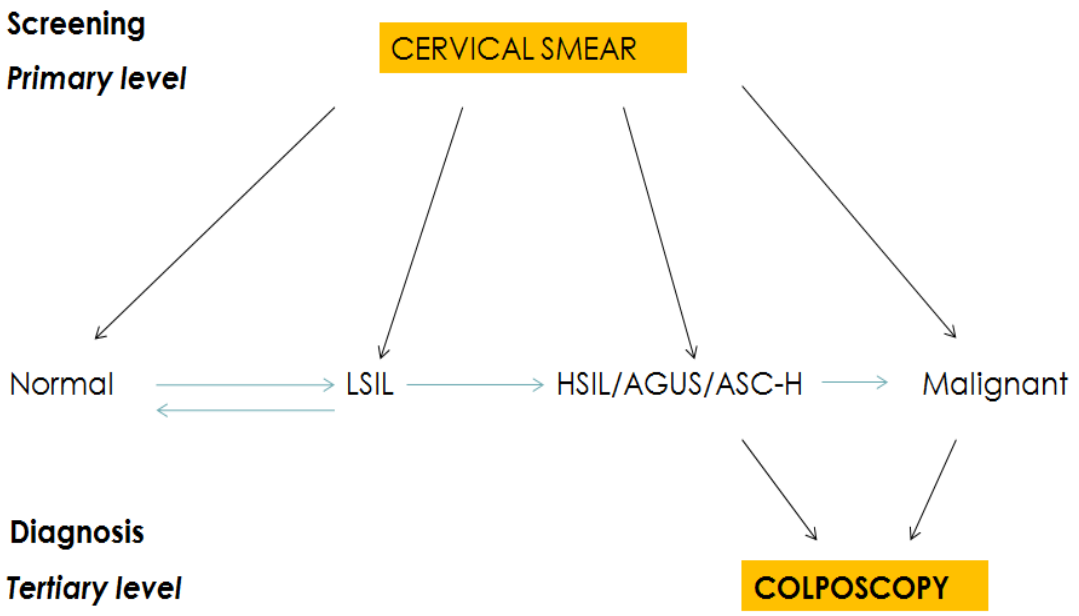

Figure 1: Figure illustrating different service levels for screening and diagnosis of cervical disease.

The World Health Organisation has guidelines for screening and treatment policies which allow decision makers to choose appropriate policies based on contextual factors[19]. In areas where there is poor follow up of patients, poor training of health care workers and low coverage of women they recommend that a screen-and-treat approach be used. In a randomised controlled trail conducted by Denny et al[20] it

was found that women who tested positive on either a HPV test (which detects HPV DNA) or visual inspection with acetic acid (VIA) (which allows lesions to be visible to the naked eye at the point of care) and were treated immediately with cryotherapy had a 77% and 37% reduction in the detection of high grade lesions at 12 months follow up, respectively, compared to women who received delayed evaluation of lesions.

Natural history of HPV

Papilloma viruses are double-stranded DNA viruses belonging to the family *Papillomaviridae*.[21] There are 29 genera made up of 180 papillomavirus types, of which 120 are human papilloma viruses. They are species-specific and therefore human papilloma viruses infect only humans. Among the different HPVs, tissue-specific tropism is displayed. Thus there are particular HPV types that infect the different tissues of the cervix, vagina, vulva, etc. Specific HPV types also infect the head and neck. Furthermore, HPV types can be considered to be either low- or high-risk, and it is this which determines whether or not infection in squamous epithelial tissue has the potential to develop into cancer. For example, HPV types 6 and 11 infect the genital tract but are low-risk types and therefore cause benign genital warts, while HPV types 16 and 18 infect the genital tract but are high-risk types and cause cervical cancer, which is a life-threatening illness.

All HPV's DNA contain 3 regions.[22] These are an upstream regulatory region, which contains genetic sequences involved in control of viral transcription and translation; an early region containing open reading frames which determine the carcinogenic properties of the virus; and a late region which contains open reading frames coding for viral structural proteins. The early genes E6 and E7, which are present in HPV 16 and 18 genomes, have been shown to bind the mammalian proteins p53[23] and pRb[24], both tumour suppressing proteins.[25, 26] Binding of these proteins sets the cell on a pathological course which ultimately results in cancer.

HPV employs a number of immune evasion mechanisms. One of these is molecular mimicry.[27] The E7 protein has been found to have homologous sequences to mammalian proteins such that antibodies produced against E7 may also target XP-G complementing protein, which is involved in repair of UV-damaged DNA.[28] It also has regions analogous to RBBP-1, which is involved in cell cycle regulation.[29] Thus, anti-E7 antibodies may also result in a dysregulated cell cycle.

Following infection with HPV, expression of MCP-1 is suppressed.[30] MCP-1 is a chemokine which attracts monocytes and macrophages, so that they are able to perform immune surveillance functions.[31] Thus by supressing MCP-1 expression, cells infected with HPV remain undetected by the immune system.

The oncoproteins E6 and E7 have also been shown to inhibit IL-8 promoter activity and thus IL-8 transcription.[32] IL-8 is a pro-inflammatory cytokine and attracts neutrophils and granulocytes to the site of infection.[33]

Thus, by using these immune evasion mechanisms, HPV may be in a cellular environment that is not reactive to the presence of virus. This is favourable for the

eventual expression of viral proteins. Specifically, once the oncoproteins E6 and E7 are expressed, the cell is set on a pathological pathway.[34]

It is unknown why some cervixes will progress to cancer and others do not. Animal models have shed light on the differences in immune responses to HPV infection in lesions that are regressing and lesions that are progressing: regressing lesions have CD4+ and CD8+ cytotoxic lymphocytes present as well as macrophages.[35] The environment is also characterised by the presence of pro-inflammatory cytokines as well as adhesion markers responsible for trafficking of lymphocytes. In contrast, lesions that are progressing have only CD8+ cytotoxic lymphocytes and mononuclear cells

HPV prevalence

Worldwide, cervical cancer is the fourth commonest cancer among all women and the second commonest among women aged 15-44 years.[36] It is estimated that roughly half of all women who develop cervical cancer will die from it. Of women with normal cervical cytology, 13.5% are positive for HPV.[36]

Infection with HPV occurs soon after sexual debut.[37] The majority of these infections are cleared spontaneously and the prevalence of HPV decreases with age, until the age of 40 and older where an increase in the prevalence among this age group is seen.[38] The factors associated with the increase in HPV prevalence seen in this older age group are unclear. Possible causes are reactivation of latent HPV infections or re-infection with HPV with increasing numbers of sexual partners.

More than half of deaths due to cervical cancer occur in the developing world.[36] Amongst African women, the prevalence of HPV is nearly two times that of the global prevalence, at 24.9%.[39] Cervical cancer is also the second commonest cancer among African women and the age-standardised mortality rate is 17.5 per 100 000 women per year.

In South Africa, the prevalence of HPV among women with normal cytology is 21%.[1] This is higher than the global average of 13.5%.

HPV and HIV Co-infection

The risk of HIV acquisition has been shown to be increased by the presence of other STIs.[40] HPV is the commonest sexually transmitted disease[41], and similarly, infection with HPV has been shown to increase the risk of infection with HIV. A meta-analysis of studies in which HIV infection following HPV infection were analysed showed that the risk of HIV infection while being positive for any type of HPV was 1.96 (CI 1.55; 2.49). For high risk HPV types, the risk of HIV infection was 1.92 (CI 1.49; 2.46).[42]

In addition, cross-sectional studies have shown that HIV positive women are more likely to be infected with HPV[43], more likely to have persistent HPV infection[44] as well as more likely to be infected with multiple HPV types.[45] HIV positive women are also more likely to have cervical cancer presursors.[46] Thus the guidelines for cervical screening in HIV positive women is slightly different to that of HIV negative

women. In South Africa, it is recommended that HIV positive women be screened for cervical cancer precursors once every three years. The Clinical Guidelines for the Management of HIV and AIDS in Adults and Adolescents[47] released in 2010 advise that HIV positive women should be screened every 3 years. The guidelines for referral follow those for HIV negative women, except that a diagnosis of normal cytology signals a repeat smear in 3 years' time and not 10 years.

Cervical cancer is classified as an AIDS-defining clinical condition.[48] That is to say that if a HIV positive woman is diagnosed with invasive cervical cancer, she would be eligible for antiretroviral therapy regardless of her CD4 count. It is the only HPV-related cancer that is considered to be AIDS defining, however other HPV-associated cancers have been found to occur with increasing frequency among HIV positive individuals compared to HIV negative individuals.[49] The use of antiretroviral therapy has been shown to decrease the risk of developing many other AIDS-defining conditions, such as Kaposi Sarcoma and non-Hodgkin lymphoma.[50, 51, 52] However, the literature regarding the effect of ARVs on cervical disease progression in HIV positive women has been controversial thus far. While some studies have shown a decreased risk for cervical disease progression, others have found no difference in the risk between HIV positive and HIV negative women, while others have found an increased risk.

Clearance of high risk HPV subtypes in women with normal cytology has been shown to be achieved in 43% and 65% of women followed up for 6 and 18 months, respectively.[53] HPV negativity in itself is a not a state which can be defined with certainty as HPV may be present at levels below the limits of detection of the test. Nevertheless, clearing an HPV infection requires cell mediated immunity.[54] In HIV positive women, immunity levels are lowered and this may explain why HIV positive women are more likely to be HPV positive than HIV negative women.

HPV vaccine

Two types of vaccines against HPV high risk subtypes have been produced (Cervarix and Guardasil[13,14]). Cervarix is a bivalent vaccine and has been shown to be highly efficacious against both incident and persistent infection with high risk HPV types 16 and 18, thus providing protection against cervical lesions.[55] Gardasil, a quadrivalent vaccine, induces immunity against infection with HPV types 6, 11, 16 and 18.[56] In addition, Gardasil has been shown to provide cross protection against HPV types not included in the vaccine.[57]

Introduction of either the quadrivalent or the bivalent vaccine into the immunisation programme of a country has been shown to result in a decrease in the prevalence of high risk HPV types as well as precancerous lesions associated with these oncogenic types.[58, 59] Furthermore, a trial in which HIV positive women have received the vaccine have shown that there is a good immunogenic response in this immunocompromised subgroup of women.[60] In addition, an immunogenic response to HPV vaccine has been shown in both HIV positive women on ART and those not on ART.[61]

The safety and immunogenicity of quadrivalent HPV vaccine has also been established in HIV positive girls between the ages of 7 and 12.[62] This is a promising finding as HPV vaccine has been found not to have an effect on clearance of existing HPV infections (and HIV positive women are more likely to be infected with HPV).[63] Thus HIV positive girls can be vaccinated along with their HIV negative counterparts, thus preventing possible infection with the relevant types once they become sexually active.

Effect of treatment of high grade lesions

Women with high grade lesions who receive treatment, where these lesions are removed, are at low risk for recurrence of these lesions. Factors associated with recurrence of these lesions are high risk HPV-positivity, lesion grade (cervical intraepithelial 2 vs 3), and margin status transformation zone type at removal of the lesion.[64] Similarly, a low risk for recurrence of high grade lesions after treatment has been found in HIV positive women.[65, 66]

Effect of ART on cervical disease in HIV positive women

Progression of the cervical cytology from normal to pathological is a process facilitated by the insertion of the HPV genome into the cellular genome and expression of the HPV genes by the cellular machinery. Oncogenic proteins encoded by the HPV genome cause the normal cellular process to be arrested and instead the cell is set on a pathogenic pathway which results in the development of a cancerous cell.[67]

This pathway that leads to a cell becoming cancerous is irreversible. Therefore, despite the fact that ARVs reverse the immunosuppressive effects of HIV, the cancerous pathway itself cannot be reversed. This may explain why some research finds that there is no difference between the incidence of cervical lesions in HIV positive women on ARVs and those not on ARVs. In fact, in this age where ARVs are accessible to many HIV positive women, one could argue that the increased longevity afforded by these drugs allows for more time for the development of cancerous cells of the cervix. Thus the importance of robust screening and diagnostic cervical services for HIV positive women is underscored.

Several studies have found that the use of ARVs result in decreased incidence of cervical lesions. Kim et al[68] found that HIV positive women on ART had a 58% reduced risk of SIL development. They also found a decreased risk for progression to SIL with increasing CD4 count. Similarly, Adler et al[69] found that women on ART with normal cervical cytology at baseline were 38% less likely to have a cervical lesion incident than those not on ART. Furthermore, they also found that women who showed regression of cervical lesions were 2.61 times more likely to be on ART.

A systematic review by Cobucci et al[70] found that ARVs increased the risk of invasive cervical cancer by 46%. The review included 5 observational studies ranging in time from 2001 to 2010.

The different outcomes in HPV clearance, SIL incidence and cervical disease progression and regression that are found in different studies are possibly due to the different baseline conditions in study participants or in the different study entrance criteria. Paramsothy et al[71] found that for women with a baseline cervical cytology of normal, ART was not associated with clearance of HPV infection. They also found that in women with LSIL or HSIL, ART was associated with HPV clearance. In addition, despite the association of ART with HPV clearance, in women with cervical dysplasia, ART was not associated with regression of these lesions.

In addition, an analysis of trends in AIDS-defining cancers in the era of ARVs found that the risk for incidence of these was still higher than in the HIV-negative population.[72] Thus in spite of the increasing accessibility of HIV positive women to therapy, it remains prudent to provide cervical screening services at a greater frequency than that for HIV negative women.

Conclusion

The effect of ARVs on the progression of cervical disease in HIV positive women has been controversial thus far. Further studies are warranted to contribute to the literature available thus far in order to optimise ARV therapy on HIV positive women, who are at higher risk of HPV positivity as well as development of cervical lesions.

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Part B: Manuscript

Comparative analysis of cervical disease progression in HIV positive women receiving ART and those not receiving ART in Cape Town, South Africa, from 2002 to 2011.

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Background: Women who are HIV positive have been found to be more likely to be infected with Human papillomavirus (HPV) than HIV negative women. HPV infection of the cervix is necessary for the development of cervical cancer, which is an AIDS-defining illness. The impact of antiretroviral therapy (ART) on the incidence of cervical cancer and its precursors is controversial. We determined the incidence of squamous intraepithelial lesions (SIL) in women receiving ART and compared it to those women not receiving ART.

Methods: 400 HIV positive women from a primary healthcare clinic and a colposcopy clinic were enrolled. Women were followed up at 6-monthly intervals at which Pap tests and colposcopy were performed, and survival analysis was used to determine the time from baseline diagnosis of normal to a diagnosis of SIL.

Results: Out of 400 women, 177 had a diagnosis of normal at the baseline visit. Follow-up was censored at the first of SIL diagnosis and receiving treatment for cervical disease. The incidence rate of SIL in women not on ART was 28.5 per 100 person years and in women who had initiated ART it was 30.82 per 100 person years. There was therefore no difference in the incidence rate of SIL between the 2 groups (RR=1.08; 95% CI 0.64-1.75).

Conclusion: Our results show no difference in the incidence rate of SIL between women on ART and women not on ART. Since ART results in an increased lifespan of HIV positive women and since these women are more likely to be HPV positive, efforts need to be taken to strengthen cervical screening and referral systems for HIV positive women in particular.

Introduction

South Africa has an estimated human immunodeficiency virus (HIV) prevalence of 10.2%, one of the highest in the world and has the largest HIV epidemic in the world, with an estimated 5.5 million people infected.[1] The prevalence of human papillomavirus (HPV) among South African women is high, with an estimated 18.4%[2] of women with normal cervical cytology being positive for HPV. The prevalence of HPV 16 or 18 among women with normal cytology, high grade lesions and cervical cancer is 3.2, 30.3 and 63.9%.[2]

Furthermore, it has also been found that women who are HIV positive are more likely to be infected with HPV, to have persistent infection with high-risk types of HPV, and to be infected with multiple types[3, 4, 5] In addition there are data to suggest that cervical cancer precursors are more frequently diagnosed in HIV positive women and progression to invasive cervical cancer is faster than in HIV negative women[6, 7].

Where successful screening programs have been implemented and sustained, as has occurred in a number of developed countries[8], cervical cancer incidence and mortality has been substantially reduced. Few developing countries have had sufficient human, financial and health infrastructure resources to either initiate or sustain cervical cancer screening programs. The HIV/AIDS epidemic has highlighted the need for cervical cancer screening in HIV positive women, particularly women on anti-retroviral therapy (ART), who are likely to live longer and therefore are at greater risk of developing cervical cancer.

The advent of the antiretroviral therapy era saw a drop in incidence and mortality due to AIDS-defining illnesses, such as Kaposi Sarcoma[9] and non-Hodgkin's Lymphoma[10]. However, the same cannot be said for cervical cancer. Various studies have yielded conflicting results regarding the association between antiretroviral therapy and cervical disease progression, and as a result it is still unclear whether the use of antiretroviral therapy results in a decrease in cervical neoplasias. Some of the evidence points to a decrease in the occurrence of cervical dysplasia [11,12], however other evidence indicates that there is no change in prevalence of SIL among HIV positive women on antiretroviral therapy.[13]

This study presents the results of a longitudinal study of cervical disease progression in a cohort of 400 HIV positive women followed up for 9 years in Cape Town, South Africa. Progression of cervical disease was compared in those women who were not on ART and those women who were on ART. We also report the effects of CD4 count on cervical disease as well as the progression of cervical disease following treatment for SIL.

Methods and Materials

The methods and materials have been presented previously[14]. Briefly, 400 HIV-positive women were recruited from a primary healthcare clinic and a colposcopy clinic between March 2002 and January 2003. Women had to be previously unscreened for cervical cytology and HIV positive to meet inclusion criteria. Ethics approval was obtained from the University of Cape Town's Research Ethics Committee. Each woman provided written informed consent and underwent the following procedures: conventional cytology sampling of the cervix for detection of high-risk HPV subtypes and colposcopy with histological sampling if appropriate. Cervical smears were analysed by the South African National Health Laboratory Service. For histologic reporting the following nomenclature was used: CIN-1 was referred to as LSIL and CIN-2/3 were grouped together and referred to as HSIL. For statistical analysis, both the colposcopic and histologic diagnosis (where available) were combined. This yielded a composite diagnosis which was used as the end point in survival analysis.

Cervical samples were collected using the Qiagen DNA collection device, (Qiagen, Gaithersburg, Inc., MD, USA), and assayed for high risk HPV using the Hybrid Capture II test (Qiagen, Gaithersburg, Inc., MD, USA). Details of the assay have been described previously[14]. Blood was taken at each visit to determine CD4 counts and HIV viral load. CD4 counts were determined every 6 months. HIV viral load was determined every 18 months for the first 2 years.

All acetowhite lesions were biopsied. Women were treated with large loop excision of the transformation zone (performed under local anaesthetic in the clinic) if they had a histologically confirmed diagnosis of HSIL.

Women who were eligible for ART initiation (i.e. women with a CD4 count below 200 cells/mm^3 or diagnosed with an AIDS-defining illness) were offered ART through primary health care facilities.

The data were analysed using StataMP 11.0 (StataCorp, College Station, TX). All statistical tests were 2-tailed at α=0.05. Survival analysis was used to determine time from a baseline diagnosis of normal to SIL. Thus, entry into the statistical study required that women be SIL-free at baseline. Women were censored at the first of SIL diagnosis and receiving treatment for cervical disease. Kaplan-Meier survival plots were generated and these were stratified by ART status and CD4 count. Risk ratios for the development of SIL are presented, by ART status, baseline CD4 count, baseline HPV status and age. Cox proportional hazards regression was also performed and hazard ratios are presented for SIL development, both crude and adjusted for ART status, CD4 count (as a time-varying covariate) and age.

Results

Study Sample and Baseline Characteristics

The baseline sociodemographic characteristics of this cohort have been described previously[14]. Table 1 shows the baseline characteristics of women who were on ART at their final visit and those who were not.

Of the 400 women, 114 (28.5%) had died and 172 (43%) were lost to follow up by the end of the follow up time. The final visit date was defined as the 18th biannual visit date. Women who had their last visit date more than 1.5 years before this date and who were not confirmed as deceased were considered to be loss to follow up.

The median CD4 count at baseline for women who had initiated ART by their final visit was 217/mm^3 compared to 268/mm^3 for women who did not start ART. Overall, 74% of all women tested positive for high risk HPV at baseline compared to 63% of women who did not start ART. The proportion of women with normal, LSIL and HSIL histological diagnoses at baseline were similar amongst those who were on ART and those not on ART at the final visit (45 vs. 43%, 40 vs. 40% and 17 vs. 15%, respectively).

Table 1: Baseline sociodemographic characteristics of women stratified by whether or not ART was initiated during follow up and further by HPV status at baseline.

	Overall (n=400)				Baseline HPV status					
					HPV Positive (n=269)			HPV Negative (n=128)		
		Initiated ART (n=168)	Did not initiate ART (n=232)	P value	Initiated ART (n=123)	Did not initiate ART (n=146)	P value	Initiated ART (n=44)	Did not initiate ART (n=84)	P value
Median age (range)	28 (18-52)	29 (18-52)	28 (18-51)	0.108	29 (18-49)	28 (18-51)	0.063	28 (19-52)	28 (18-50)	0.964
Median CD4 count	248	217	268	0.009	207	219	0.775	259	374	0.002
CD4 200 or less (%)	158 (40)	76 (45)	82 (35)		59 (48)	67 (46)		16 (36)	14 (17)	
CD4 201-500 (%)	185 (46)	80 (48)	105 (45)		56 (45)	60 (41)		24 (55)	44 (52)	
CD4 more than 500 (%)	57 (14)	12 (7)	45 (19)		8 (7)	19 (13)		4 (9)	26 (31)	
Median log HIV viral load (copies/ml)	4.32	4.43	4.20	0.152	4.53	4.47	0.862	4.26	3.64	0.033
Prevalence of HR-HPV (n=397) (%)	269 (67)	123 (74)	146 (63)	0.032						
Ever smoked (%)	47 (12)	18 (11)	29 (13)	0.574	15 (12)	17 (12)	0.889	3 (7)	12 (14)	0.204
Median age of first intercourse (range)	16.9 (9-26)	17 (12-25)	17 (9-26)	0.112	17 (12-25)	16.5 (9-21)	0.190	17 (14-20)	17 (13-20-	0.422
Number of sexual partners										

• **1-5**	198 (50)	130 (77)	168 (72)	0.500	93 (76)	106 (73)	0.948	36 (81)	60 (71)	0.228
• **More than 5**	102 (26)	38 (23)	64 (28)		30 (24)	40 (27)		8 (18)	24 (29)	
Currently sexually active	299 (75)	130 (77)	169 (73)	0.303	94 (76)	109 (75)	0.737	35 (80)	59 (70)	0.257
Consistent condom use	115 (29)	49 (38)	66 (39)		41 (44)	46 (42)		8 (23)	20 (34)	
Baseline diagnosis										
• **Normal**	177 (44)	72 (43)	105 (45)	0.827	36 (30)	47 (32)	0.775	35 (80)	58 (69)	0.02
• **LSIL**	160 (40)	67 (40)	93 (40)		63 (52)	69 (47)		4 (9)	23 (27)	
• **HSIL**	62 (16)	28 (17)	34 (15)		23 (19)	30 (20)		5 (11)	3 (4)	

ART Initiation

Over one quarter (26.8%) of the participants were lost to follow up at 6 months into the study (i.e. at the second visit). Following this there is gradual increase in loss until 56.3% were lost to follow up by the 7th visit. The greatest loss between two visits occurs between the 17th and 18th visit dates (i.e. between the penultimate and last visits), where 58.1% of participants were lost. This is followed by the initial loss of 26.8% of the participants by the second visit, and then 19% between visits 6 and 7. By the final visit date, 90.3% of the participants were lost to follow up. By the end of the 9 year follow up period, 42% of the 400 women had accessed ART and 12% had received treatment for cervical lesions.

Table 2: Number of women at each visit, number of women starting ART at each visit and number of women receiving treatment for cervical disease at each visit

Month since enrolment into study	Number completing visit	Percentage drop out	Cumulative percentage drop out	Number starting ART at visit	Total number on ART at visit	Cumulative started ART	Number treated at visit	Cumulative treated
0	400			0	0	0	8	8
6	293	26.8	26.8	7	7	7	11	19
12	288	1.7	28.0	17	24	24	4	23
18	260	9.7	35.0	17	39	41	9	32
24	242	6.9	39.5	22	56	63	1	33
30	224	7.4	44.0	26	79	89	0	33
36	216	3.6	46.0	27	102	116	3	36
42	175	19.0	56.3	17	103	133	2	38
48	165	5.7	58.8	8	105	141	1	39

54	149	9.7	62.8	4	98	145	1	40
60	142	4.7	64.5	1	91	146	1	41
66	137	3.5	65.8	2	93	148	1	42
72	116	15.3	71.0	5	82	153	0	42
78	110	5.2	72.5	3	79	156	1	43
84	101	8.2	74.8	2	75	158	0	43
90	99	2.0	75.3	5	77	163	1	44
96	96	3.0	76.0	0	75	163	2	46
102	93	3.1	76.8	4	76	167	0	46
108	39	58.1	90.3	0	34	167	0	46

Fifty per cent of the women had initiated ARV treatment by 1281 days or 3.5 years, which corresponds to the eighth six-monthly visit.

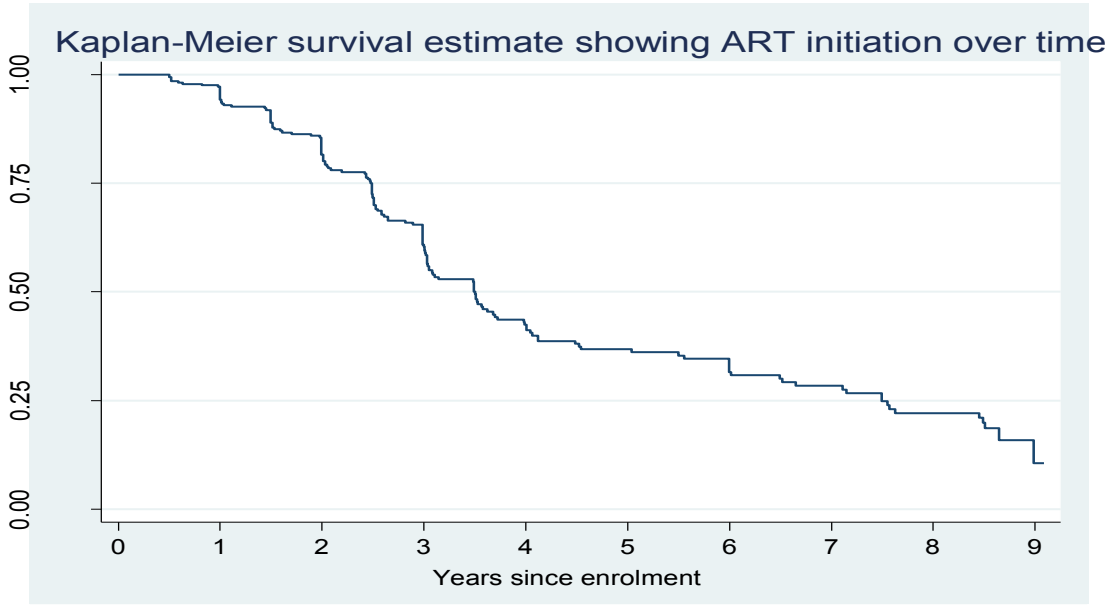

Figure 1: Kaplan Meier plot showing ART initiation over time.

CD4 Counts and SIL Development

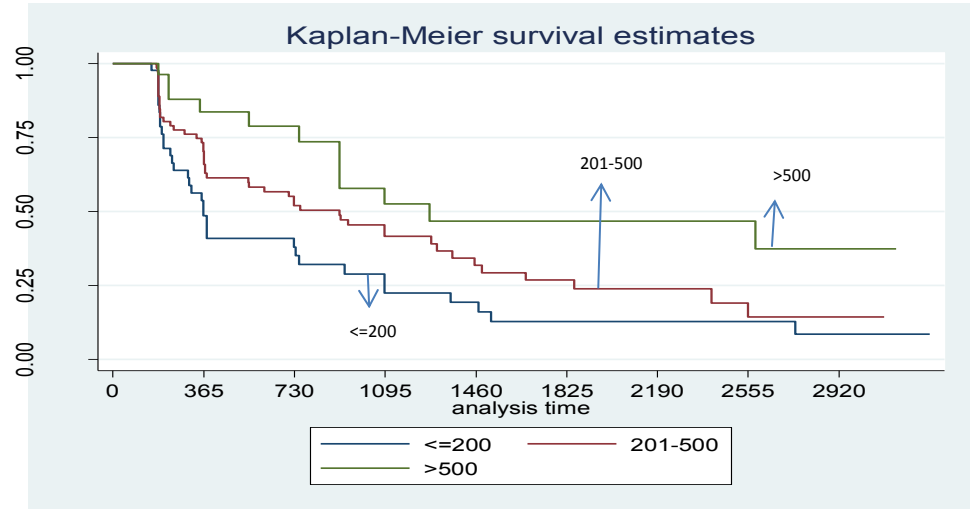

Figure 2: Kaplan Meier plot showing SIL development over time, stratified by baseline CD4 count. CD4 counts shown are in cells/mm^3.

The Kaplan Meier plot in figure 2 shows that there is an increase in the rate of progression to SIL with decreasing baseline CD4 count. Compared to having a baseline CD4 count of 200 cells/mm^3 or less, the rate ratio of progression to SIL in those who had a baseline CD4 count of 201-500 cells/mm^3 was 0.65 (95% CI 0.41-1.04). For those with a baseline CD4 count greater than 500 cells/mm^3 the rate ratio was 0.33 (95% CI 0.16-0.66) compared to the lowest CD4 category. Thus having a

baseline CD4 count of 201-500 and greater than 500 cells/mm^3 was 35 and 67% protective against the development of SIL, respectively.

Among women who initiated ART, the lowest CD4 counts ranged from 5 to 510 cells/mm^3, with a median CD4 count of 104 cells/mm^3. For women who did not initiate ART, the lowest CD4 counts ranged from 4 to 1037 cells/mm^3, with a median CD4 count of 179 cells/mm^3. Six months prior to starting ART, 59, 34 and 7% of women had a diagnosis of normal, LSIL and HSIL, respectively.

Those who died or were lost to follow up had similar baseline characteristics to those who were retained, apart from the median CD4 count and whether or not they were on ART at the final visit. In addition, there was a difference in the median viral load between those retained and those who died.

Table 3: Comparison of baseline characteristics of women who died, were lost to follow-up and retained in the cohort.

	Retained (n=114)	**Died (n=114)**	**p value***	**LTFU (n=172)**	**p value****
Mean age (range)	29.6 (18-49)	29.3 (18-50)	0.745	29.0 (18-52)	0.430
Median CD4 count	264	130.5	<0.0001	305	0.123
CD4 200 or less (%)	40 (35.1)	75 (65.8)		43 (25.0)	
CD4 201-500 (%)	58 (50.9)	33 (29.0)		94 (54.7)	
CD4 more than 500 (%)	16 (14.0)	6 (5.3)		35 (20.4)	
Median log HIV viral load (copies/ml)	4.26	4.81	<0.0001	4.04	0.646
Prevalence of HR-HPV (%)	76 (66.7)	82 (72.6)	0.334	111 (65.3)	0.811
Ever smoked (n=399) (%)	12 (10.5)	13 (11.4)	0.832	22 (12.9)	0.551
Mean age of first intercourse (range)	17 (13-23)	16.8 (11-26)	0.468	16.9 (9-25)	0.753
Number of sexual partners					
• **1-5**	87 (76.3)	84 (73.7)	0.646	127 (73.8)	0.636
• **More than 5**	27 (23.7)	30 (26.3)		45 (26.2)	
Currently sexually active (%)	85 (74.6)	76 (66.7)	0.191	138 (80.2)	0.257
Consistent condom use (%)	33 (38.8)	35 (46.1)	0.833	47 (34.3)	0.212

On ART (at final visit)	91 (79.8)	12 (10.53)	<0.0001	63 (36.6)	<0.0001

LTFU was defined as those who had their last visit date more than 1.5 years from the last date in the study and were not recorded as dead.

*P values are for comparisons of Died to Retained

**P values are for comparisons of LTFU to Retained

Effect of ART on Incidence of SIL

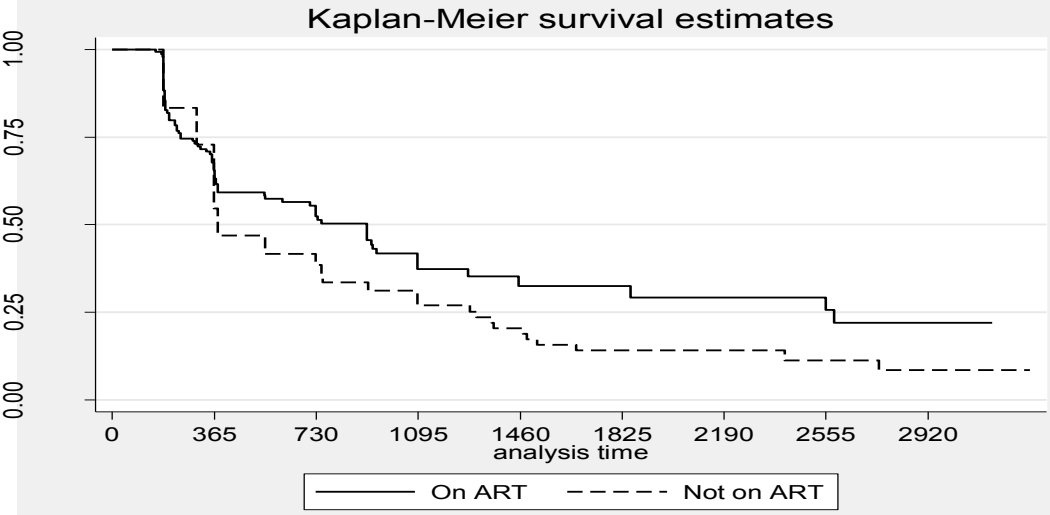

Figure 3: Kaplan-Meier analysis of SIL-free survival in women on or not on ART. p=0.132

Figure 3 shows the Kaplan Meier plot for SIL-free survival, stratified by ART status. Women with a baseline diagnosis of normal were included in the analysis. This included 177 out of the 400 women. Women were censored from the analysis upon receiving treatment for cervical disease. The total person time at risk was 352 person years. The incidence rate of SIL in women not on ART was 28.5 per 100 person years and in women who had initiated ART it was 30.82 per 100 person years. The rate ratio was therefore 1.08 (95% CI 0.64-1.75).

For women with a baseline viral load greater than log 5, there was a 25% increase in the incidence of SIL compared to women with a baseline viral load below log 5 (rate ratio 1.25 95% CI 0.78-1.96). Women who had baseline CD4 counts greater than 500 cells/mm^3 at baseline had a 67% reduction against the incidence of SIL and those with CD4 counts of 201-500 cells/mm^3 had a 37% reduction in the incidence of SIL compared to women with CD4 counts of 200 cells/mm^3 and below (rate ratio 0.36 95% CI 0.18-0.68; 0.63 95% CI 0.40-0.99; respectively). Baseline HPV positivity was the strongest predictor of progression to SIL. Women who were positive for high risk HPV at baseline had an incidence rate of SIL 2.46 times higher than women who were HPV negative at baseline (95% CI 1.63-3.74). There was no difference in the incidence of SIL in women aged 30-39 years compared to women

aged 29 years and younger (rate ratio 1.05 95% CI 0.67-1.64). However women who were aged 40 years and older had a 58% reduction in the incidence of SIL compared to the youngest age group (rate ratio 0.42 95% CI 0.16-0.91).

Table 4 shows the Cox proportional hazards models for incidence of SIL. Overall, the crude hazard ratio for incidence of SIL for those on ART compared to those not on ART was 1.3 (95% CI 0.5-3.2). After adjusting for CD4 count and age, the hazard ratio was 0.9 (95% CI 0.4-2.5), showing no difference in incidence of SIL between those on ART and those not on ART. The crude hazard ratios showed that having a CD4 count above 200 cells/mm^3 and being 40 years and older were protective against incidence of SIL (HR 0.5 95% CI 0.3-0.9; HR 0.5 95% CI 0.2-1.1; respectively). Adjusted for ART, CD4 count and age, a higher CD4 count and older age were still protective.

Disease progression to a diagnosis of SIL was also determined from the time of treatment of cervical disease. The incidence rate of SIL following treatment was 28.5 per 100 person years. For those women on ART and not on ART, the incidence rate was 24.7 and 33.6 per 100 person years, respectively. The incidence rate ratio was thus 0.74 (95% CI 0.2-2.75).

Table 4: Cox proportional hazards models showing ART and incidence of SIL, presented as hazard ratios with 95% confidence intervals.

	Overall		**Pre-ART**		**On-ART**	
	Crude	**Adjusted**	**Crude**	**Adjusted**	**Crude**	**Adjusted**
	HR (CI)	HR (CI)	HR (CI)	HR (CI)	HR (CI)	HR (CI)
On ART	1.3 (0.5-3.2)	0.9 (0.4-2.5)	-	-	-	-
CD4 count						
≤200*	-	-	-	-	-	-
>200	0.5 (0.3-0.9)	0.5 (0.2-0.9)	0.5 (0.3-1.0)	0.5 (0.2-0.9)	0.2 (0.02-2.1)	0.3 (0.02-2.9)
Age						
≤29†	-	-	-	-	-	-
30-39	1.1 (0.7-1.6)	0.9 (0.6-1.5)	1.2 (0.7-1.9)	1.1 (0.7-1.9)	0.4 (0.1-1.0)	0.4 (0.1-1.1)
≥40	0.5 (0.2-1.1)	0.4 (0.2-0.9)	0.6 (0.3-1.6)	0.5 (0.2-1.2)	0.1 (0.03-0.7)	0.1 (0.03-0.8)

Crude hazard ratios are not adjusted for any other covariates. Adjusted hazard ratios are adjusted for all covariates listed.

*Reference category for CD4 count.

†Reference category for age.

Discussion

Cervical cancer remains one of the commonest forms of cancer in women in developing countries, including South Africa. The crude incidence and age-standardised mortality rates for cervical cancer are 30.2 and 18 per 100 000, respectively[2]. Cervical disease is progressive and develops over many years, involving the development of squamous intraepithelial lesions which can be detected by Pap smears and for which treatment is available. In HIV positive women, this progression of cervical disease occurs more rapidly[15,16]. This study examined the incidence of SIL development in HIV positive women and determined the incidence rate ratio between women on ART and women not on ART.

Women were followed up for 9 years. Follow up was censored at the first of either SIL diagnosis or treatment for cervical disease by LLETZ (large loop excision of the transformation zone). Our primary finding was that there was no difference in the risk for SIL development between women receiving ART and women not receiving ART. The incidence rate in women on ART was 28.5 per 100 person years compared to an incidence rate of 30.82 per 100 person years in women not on ART. The rate ratio in our cohort is therefore 1.08 (95% CI 0.64-1.75).

Several studies have found conflicting results with respect to the effect of ART on cervical disease progression. A study conducted in Soweto, South Africa found that ART was protective against SIL development (HR 0.72; 95% CI 0.52-0.99)[17]. However, this study reported cervical diagnoses based on cervical smears (i.e. cytological results) whereas we report histologic results. However, one of their findings was that older age was protective against disease progression compared to the lowest age category (18-25 years), and in fact they found that older age was associated with regression of cervical disease. This is in agreement with our finding of a 58% decrease in cervical disease progression in women older than 40 compared to women younger than 40.

Similarly, a cross-sectional study found that there was no statistical relationship between ART use and cervical disease[18]. However, because the study was a cross-sectional study it is only possible to comment on prevalence and not incidence of cervical disease progression.

Heard et al[19] found that the prevalence of SIL among HIV positive women decreased significantly after a median period of 5 months after starting ART. A cohort study by Minkoff et al[20] found that women on ART were 32% less likely to have progression of cervical disease over 6 months. Soncini[21] et al found that over a 37 month follow up period, women on ART had a 70% reduction in the risk for developing CIN compared to HIV positive women not on ART, over the same time period. On the other hand, monitoring of the rate of progression of cervical disease in 163 HIV positive women over a mean period of 15.4 months[13] showed that there was no difference between women on ART and women not on ART. In addition, Schuman et al[22] reported that ART-use did not result in a decreased risk of incident SIL.

Approximately one third of the colposcopy diagnoses also had a histopathology result. From these two, a composite diagnosis was derived and this was used in all

analyses. Where histopathology was not available, the visual colposcopy diagnosis was used. As far as we know this is the only cohort study which uses a composite diagnosis (where it is available) in analysis. This is a much more reliable reading as visual inspection of the cervix by colposcopy is sensitive to the subjectivity of the interpreter.

Sahasrabudde et al used a composite diagnosis in a cross-sectional study[23] and found an increase in the odds of HSIL (CIN2 and CIN3) for women who were currently receiving ART compared to those who had never received ART (OR=2.24; 95% CU 1.17-4.26).

We found that immunological status was a predictor of SIL development, specifically that a baseline CD4 count greater than 500 cells/mm^3 resulted in a 67% lowered likelihood of progression from a baseline cytological diagnosis of normal to a diagnosis of SIL, compared to a reference group whose baseline CD4 counts were 200 cells/mm^3 or less. Similarly those with baseline CD4 counts between 201 and 500 cells/mm^3 had a 35% reduction in progression to SIL, compared to the reference group. This is in agreement with findings of 2 other cohort[24, 25] studies that used survival analysis to show that baseline CD4 counts greater than 200 cells/mm^3 were protective against SIL development.

Thus far the study of the effect of ART on cervical disease progression in HIV positive women has been controversial. Several different studies have yielded results that ranged from a decrease in the development of SIL to no difference in the outcomes between those on ART compared to those not on ART. Our study has shown no difference in the hazard of SIL development between ART use and ART non-use. A possible reason for the different results obtained could be the study inclusion criteria. Soncini et al[26] excluded women with a previous history of cervical intraepithelial neoplasia, and they found a 70% reduction in cervical disease in those on ART compared to those not on ART. In our study, history of neoplasia was not an exclusion criterion. This difference could result in an underestimation of the measure of effect in our study as women in the study may already have lesions that are somewhere along the continuum of cervical disease progression. This is consistent with the theory on the genetic alteration of the cells within a lesion. Once the cell has altered genetically, it cannot revert to its previous state of normality. ART is initiated based on immunological status. However, immune reconstitution based on the use of ART cannot cause the cell to go back to its normal state and thus it will continue to progress pathologically[27].

In our study the women served as their own controls, in that the time a participant spent in the study before initiating ART was included in the analysis time of ART non-use. This decreases the variance in the measurements taken in the study. A limitation of the study is that we did not have an HIV negative group whose disease progression could have been compared to those participants with CD4 counts above 500 cells/mm^3.

The loss to follow up in our study was high. By the end of the 9-year period, 90% of the study participants were lost to follow up. Nearly one third (29.3%) of the women died during the course of the study. At the time, ART eligibility required a CD4 count of 200 cells/ml. Thus, these women were more ill than if they were to be initiated at current ART initiation guidelines. This may have resulted in the high rate of death.

The high numbers lost to follow up and to death could have resulted in an underestimation of the measure of effect, as a greater number of study participants for a longer period of time may be correlated with an increase in the observations of incident SILs.

ART use is being scaled up in South Africa and therefore the lives of HIV positive women will be prolonged. We found no difference in the risk of SIL development in HIV positive women who are on ART and those who have not yet initiated ART. In light of this it is important that cervical screening and referral systems for colposcopy are strengthened to meet an important need.

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Part D: Appendix

Table of Contents

Ethics Approval Form

UNIVERSITY OF CAPE TOWN

Department of Obstetrics and Gynaecology

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HEAD of DEPARTMENT: Professor Lynette Denny **MBChB (UCT), MMed(O&G), PhD, FCOG(SA)**
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HUMAN RESEARCH ETHICS COMMITTEE
-7 SEP 2012
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

29 August 2012

Prof Blockman
Research Ethics Committee
Faculty of Health Sciences
University of Cape Town

Dear Prof Blockman

Re: Update for study Management of Abnormal Cytology in HIV-1 Infected Women (Mach-1) and Request for reapproval.
Rec Ref: 106/2002

The prospective study of 400 untreated, HIV-1 infected women, undergoing high-risk (HR)-HPV DNA testing (Hybrid Capture II™), cytology, colposcopy, histology and CD4 count testing, 6 monthly for 36 months, for 60 months and extended for a further 5 years is on-going. Re-approval for a further year is requested.

The study has been conducted to examine the natural history of HR-HPV infection and cervical disease in HIV-1-infected women. In addition, the study aims to determine the HR-HPV type distribution among HIV-positive women to allow an estimation of the potential impact of HPV vaccines in HIV-positive women.

Information regarding the follow-up of patients is as follows:
VISIT 108: 91 subjects attended V108
VISIT 114: Is ongoing and 81 subject have attended thus far
VISIT 120: Is ongoing and 36 subjects have attended thus far

FACULTY OF HEALTH SCIENCES UCT
HUMAN RESEARCH ETHICS COMMITTEE
CORRESPONDENCE NOTED AND FILED
COMMENT:
Signature removed 8/9/2012
ETHICS COMMITTEE CHAIR _____ DATE _____

Deceased: 112 subjects
Relocated permanently: 34 subjects
Untraceable: 69 Subjects

Yours sincerely
Signature removed
Lynette Denny

Consent Form

Consent to Participate in study of the Management of Abnormal Cytology in Women infected with HIV-1.

I.....agree to participate in the study designed to study the management of women who are infected with HIV and who have abnormal Pap smears. I understand that my participation in the study is voluntary and I can withdraw from the study at any point and that withdrawal from the study will not impact on my clinical care.

. I understand that all information, including my HIV status will be completely confidential and that this information will be kept in my confidential hospital file. If I agree to undergo HIV testing and I am HIV positive, I agree that the following tests may be performed on me on a six monthly basis:

- A Pap smear
- A colposcopic examination (examination of the cervix after the application of acetic acid (vinegar) to the cervix and histological sampling (removal of 1-2 mm of tissue from the cervix)
- A test for infection with the Human Papillomavirus
- HIV viral load (the amount of virus in the blood)
- CD4 counts (the white blood cells that are affected by the HIV virus)

I understand that any abnormalities detected during the course of the study will be managed and treated according to the routine protocol of the clinic.

I further understand that all results of tests performed on me will be given to me and their meaning fully explained to me. In addition, results of my tests will be entered into a confidential computerized data base, and I will be identified in this data base by my unique study number, not by name.

Signed ..... Printed Name.....

Hospital Folder Number (sticker).

Study Number .....

Date.....

Witness .....

Registration Forms

Demographic Information

Demographic information — Registration Form

Please circle:

Centre: 1 Edinburgh; 2 London Chelsea; 3 London, Kings College; 4 London, St. Thomas; 5 London, St. Mary's; 6 Manchester; 7 Milan; 8 Paris; 9 Dublin; 10 Warsaw; M South Africa

Patient number

Date of birth d d m m y y

Date of enrolment d d m m y y

Origin: European/North; X Sub-Saharan Africa; Other (Specify) MACH-TRIAL

Details of infection

Date of 1st positive HIV test d d m m y y

Route of infection: IDU; Heterosexual; Blood; Unknown (*if IDU H enter as IDU*)

Sexual history

Age at 1st intercourse

Number of sexual partners: 1; 2-5; 6-20; >20

In the last 6 months

Heterosexually active: Yes; No

Number of male partners; Declined (✓)

Frequency of condom use: <20%; 20-60%; 60-90%; Always

Smoking

Ever; Never

In the last month: No; Yes; Number per day

Laboratory tests

	Date	Result
CD4		
HIV viral load	d d m m y y (If different from CD4 date)	

Antiretroviral treatment (check actual treatment) On treatment: Yes; No

CYTOLOGY | Previous abnormal ☐ Yes ☐ No ☐ Never screened ☐ Unknown

	Date	ASCUS	LGSIL	HGSIL	INVAS	INAD	NORMAL
Earliest abnormal							
1st worst							
Most recent result							

COLPOSCOPY Previous colposcopy ☐ Yes ☐ No ☐ Unknown

	COLPOSCOPY					HISTOLOGY					
	Date	Normal	Low Grade	High Grade	Cancer	Size of lesion	Punch biopsy (y/n)	Kolio/CIN1	CIN2	CIN3	Cancer	Normal
"earliest abnormal"												
"1st worst"	d d m m y y											
"most recent result"	d d m m y y											

TREATMENTS Previous treatments ☐ Yes ☐ No If yes, number ☐

	TREATMENT TYPE				HISTOLOGY					
	Date	Loop	Laser	Knife	Margins clear y/n	Kolio/CIN1	CIN2	CIN3	Cancer	Normal
Earliest treatment										
Most radical treatment	d d m m y y									
Most recent treatment	d d m m y y									

HPV TESTING Previous HPV testing ☐ Yes ☐ No *(If yes, please give details of 3 most recent tests below)*

	Type of test (✓)			Result						
Date of test	HC2	PCR	SB	HR (✓)	LR (✓)	Types				
d d m m y y										
d d m m y y										
d d m m y y										

Initial Examination

FIRST EXAMINATION Enter date if different from registration date ☐

Condylomata present ☐ Yes ☐ No

Cytology performed ☐ Yes ☐ No

Result

Date	ASCUS	LGSIL	HGSIL	INVAS	INAD	NORMAL
d d m m y y						

Colposcopy performed ☐ Yes ☐ No ☐ Satisfactory ☐ Unsatisfactory

COLPOSCOPY

HISTOLOGY

Date (if different from above)	Normal	Low Grade	High Grade	Cancer	Size of lesion	Punch biopsy (y/n)	Kolio/CIN1	CIN2	CIN3	Cancer	Normal
d d m m y y											

TREATMENTS ☐ Yes ☐ No

TREATMENT TYPE

HISTOLOGY

Date (if different from above)	Loop	Laser	Knife	Margins clear y/n	Kolio/CIN1	CIN2	CIN3	Cancer	Normal
d d m m y y									

HPV TESTING ☐ Yes ☐ No

Date of test (if different from above)	Type of test (✓) HC2	PCR	SB	HR (✓)	LR (✓)	Result Types
d d m m y y						

3.

Follow up visits

Demographic Information

Demographic information — Follow-up visit

Visit Number: ☐☐

Please circle:

Centre:
1 Edinburgh
2 London Chelsea
3 London, Kings College
4 London, St. Thomas
5 London, St. Mary's
6 Manchester
7 Milan
8 Paris
9 Dublin
10 Warsaw
✓ South Africa

Patient number ☐☐☐☐

Date of birth ☐☐/☐☐/☐☐☐☐

Date of enrolment ☐☐/☐☐/☐☐☐☐

Since last visit

Heterosexually active ☐ Yes ☐ No

Number of male partners ☐ ☐ Declined (✓)

Frequency of condom use ☐ <20% ☐ 20-60% ☐ 60-90% ☐ Always

Smoking (since last visit)

☐ No ☐ Yes Number per day ☐

Laboratory tests

	Date	Result
CD4	☐☐/☐☐/☐☐☐☐	☐☐☐☐
HIV viral load	☐☐/☐☐/☐☐☐☐ (if different from CD4 date)	☐☐☐☐☐

Antiretroviral treatment (check actual treatment) On treatment ☐ Yes ☐ No

Examination details

EXAMINATION (VISIT NUMBER) ☐ PATIENT NUMBER ☐☐☐

Condyloma present ☐ Yes ☐ No

Cytology performed ☐ Yes ☐ No

Date	Result						
	ASCUS	LGSIL	HGSIL	INVAS	INAD	NORMAL	

Colposcopy performed ☐ Yes ☐ No ☐ Satisfactory ☐ Unsatisfactory

COLPOSCOPY

Date (if different from above)	Normal	Low Grade	High Grade	Cancer	Size of lesion	Punch biopsy (y/n)

HISTOLOGY

Kolio/CIN1	CIN2	CIN3	Cancer	Normal

TREATMENT ☐ Yes ☐ No

TREATMENT TYPE

Date (if different and above)	Loop	Laser	Knife	Margins clear y/n	Kolio/CIN1	CIN2	CIN3	Cancer	Normal

HISTOLOGY

HPV TESTING ☐ Yes ☐ No

Type of test (✓)			Result		
HC2	PCR	SB	HR (✓)	LR (✓)	Types

2

Information for Authors

The Lancet Oncology **publishes interesting and informative reviews on any topic connected with oncology, and considers any original research contribution that advocates change in, or illuminates, oncological clinical practice. Manuscripts must be solely the work of the author(s) stated, must not have been previously published elsewhere, and must not be under consideration by another journal. All papers should be written to be clearly understandable to the journal's readers in a wide range of specialties and countries.**

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1. Covering letter
2. Manuscript including tables and panels
3. Figures
4. Author statement form (see next section)
5. Declaration of interests and source of funding statements (see next section)
6. In-press papers—one copy of each with acceptance letters
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ICMJE Recommendations
http://www.icmje.org

Author statement form
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Red section (Articles and Meta-analyses)

Articles

- *The Lancet Oncology* prioritises reports of original research that are likely to change clinical practice or thinking about oncology
- We invite submission of all clinical trials, whether phase 1, 2, 3, or 4 (see *Lancet* 2006; **368:** 827–28). For phase 1 trials, we especially encourage those of a novel substance for a novel indication, if there is a strong or unexpected beneficial or adverse response, or a novel mechanism of action
- We require registration of all interventional trials, whether early or late phase, in a primary register that participates in WHO's International Clinical Trial Registry Platform (see *Lancet* 2007; **369:** 1909–11). We also encourage full public disclosure of the minimum 20-item trial registration dataset at the time of registration and before recruitment of the first participant (see *Lancet* 2006; **367:** 1631–35. The registry must be independent of for-profit interest
- Reports of randomised trials must conform to CONSORT 2010 guidelines, and should be submitted with their protocols
- All reports of randomised trials should include a section entitled Randomisation and masking, within the Methods section
- Cluster-randomised trials must be reported according to CONSORT extended guidelines
- Randomised trials that report harms must be described according to extended CONSORT guidelines
- Studies of diagnostic accuracy must be reported according to STARD guidelines
- Observational studies (cohort, case–control, or cross-sectional designs) must be reported according to the STROBE statement, and should be submitted with their protocols
- We encourage the registration of all observational studies on a WHO-compliant registry (see Lancet 2010; 375: 348)
- Genetic association studies must be reported according to STREGA guidelines
- To find reporting guidelines see http://www.equator-network.org
- Please be aware it is *The Lancet Oncology*'s standard practice to commission an independent Comment to accompany all published Articles and Meta-analyses to add context and insight

All Articles should, as relevant:

- Be up to 3000 words with 30 references (the word count is for the main body of text only and does not include the abstract, figure or table legends, acknowledgments and conflicts of interest paragraphs, or references)
- Include an abstract (semistructured summary), with five paragraphs (Background, Methods, Findings, Interpretation, and Funding), not exceeding 300 words. Our electronic submission system will ask you to copy and paste this section at the "Submit Abstract" stage
- For randomised trials, the abstract should adhere to CONSORT extensions: abstracts (see *Lancet* 2008; **371:** 281–83)
- For intervention studies, the abstract should include the primary outcome expressed as the difference between groups with a confidence interval on that difference (absolute differences are more useful than relative ones). Important secondary outcomes can be included as long as they are clearly marked as secondary
- Use the SI system of units and the recommended international non-proprietary name (rINN) for drug names. Ensure that the dose, route, and frequency of administration of any drug you mention are correct
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- Include any necessary additional data as part of your EES submission
- All accepted Articles should include a link to the full study protocol published on the authors' institutional website (see *Lancet* 2009; **373:** 992 and *Lancet* 2010; **375:** 348)

Putting research into context

- From Aug 1, 2010, authors are invited to submit their research papers with a section in the Discussion that puts the results into context with previous work (see *Lancet* 2010; **376:** 10–11). Authors should provide a panel explaining in brief how they arrived at their bottom line message
- The Discussion section should contain a full description and discussion of the context. Authors are also invited to either report their own, up-to-date systematic review or cite a recent systematic review of other trials, putting their trial into context of the review
- The Discussion should contain a Research in context panel (see below)

Research in context

Systematic review

This section should include a description of how authors searched for all the evidence. Authors should also say how they assessed the quality of that evidence—ie, how they selected and how they combined the evidence

Interpretation

Authors should state here what their study adds to the totality of evidence when their study is added to previous work

Human Gene Organisation
http://www.genenames.org/

MIAME guidelines
http://www.mged.org/Workgroups/MIAME/miame_checklist.html

Array and GEO
http://www.ebi.ac.uk/microarray-as/ae/
http://www.ncbi.nlm.nih.gov/geo

WHO's International Clinical Trial Registry Platform
http://www.who.int/ictrp/network/trds/en/index.html

CONSORT 2010 guidelines
http://www.consort-statement.org/consort-statement/overview0/

CONSORT extended guidelines
http://www.consort-statement.org/extensions/extensions/

STARD guidelines
http://www.stard-statement.org/

STROBE statement
http://www.strobe-statement.org/

STREGA guidelines
http://www.medicine.uottawa.ca/public-health-genomics/web/eng/strega.html

To find reporting guidelines, see
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Meta-analysis

PRISMA guidelines
http://www.prisma-statement.org/

- In general, these should follow the PRISMA guidelines
- Manuscripts should be structured around five sections: Summary, Introduction, Methods, Results, and Discussion
- Aim for a maximum length of about 3000 words and 75 references
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- At the Editor's discretion, commentaries may be shortened in the interests of space
- The place to respond to something we have published is in our Correspondence section
- See Conflicts of Interest guidelines for comments

Cancer and Society

- Reviews of books and other media are often commissioned, but suggestions for contributions are welcome

Correspondence

- We welcome correspondence on content published in *The Lancet Oncology* or on other topics of interest to our readers
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Green section (Reviews, Historical Reviews, Personal Views, etc)

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- A 150-word unstructured summary should be included. Use of up to 5–6 illustrations is encouraged to aid the reader
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Search strategy and selection criteria

References for this Review were identified through searches of PubMed with the search terms "young onset", "early onset", "presenile", and "dementia" from 1990 until April, 2010. Articles were also identified through searches of the authors' own files. Only papers published in English were reviewed. The final reference list was generated on the basis of originality and relevance to the broad scope of this Review

- Systematic reviews should be prepared according to the PRISMA guidelines

Historical Review

- These should follow the same guidelines as for a Review, but should cover the chronological developments in an important or interesting area of oncology

Personal View

- These should be 2000–4000 words in length, with a maximum of 75 references

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Formatting guidelines

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- Type a single space at the end of each sentence
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References

- Cite references in the text sequentially in the Vancouver numbering style, as a superscripted number after any punctuation mark. For example:
  "...as reported by Saito and colleagues.[35]"
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  "...15[tab]Saito N, Ebara S, Ohotsuka K, Kumeta J, Takaoka K. Natural history of scoliosis in spastic cerebral palsy. *Lancet* 1998; **351:** 1687–[en rule]92."
- Give any subpart to the title of the article. Journal names are abbreviated in their standard form as in Index Medicus
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- Exact p values should be provided, unless p<0·0001

Drug names

- Recommended international non-proprietary name (rINN) is required

References

- Vancouver style—eg,

—Smith A, Jones, B, Clements S. Clinical transplantation of tissue-engineered airway. *Lancet* 2008; **372:** 1201–09.

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—Hourigan P. Ankle injuries. In: Chan D, ed. Sports medicine. London: Elsevier, 2008: 230–47.

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- All video files can be submitted alongside your article in EES

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